Modification of Plasmid and Bacteriophage DNA by Aromatic Amines: Effects on Survival, Template Activity, and Mutagenicity

Charles M. King, Mei-Sie Lee, Richard F. Jones, and Nobuya Tamura

Michigan Cancer Foundation, Detroit, Michigan

The carcinogenic and mutagenic effects of the aromatic amines are believed to depend on their covalent modification of DNA, primarily through the formation of adducts at C8 of guanine. The actual biologic and biochemical responses to these adducts can be envisioned as the consequence of the abilities of the cell to repair the lesions, with or without fidelity, and the introduction of errors through bypass of the adducts by polymerases. A key question is whether changes in DNA sequence arise through the participation of common repair processes that cause mutations independent of adduct structure. Alternatively, do mutations arise through miscoding during polymerase bypass at the site of the adducts and are, therefore, more likely to produce sequence changes that are more characteristic of adduct structure? This question has been approached using single, site-specific, or randomly introduced aromatic amine DNA adducts in bacterial cells, and *in vitro* studies with DNA polymerases that employ site-specifically modified templates. The results of both approaches demonstrate that these adducts are distinguished readily by virtue of their structures, thus supporting the conclusion that mutagenic effects of the aromatic amines arise from their structures rather than from their triggering a common inaccurate repair response. —Environ Health Perspect 102(Suppl 6):217–220 (1994)

Key words: adducts, aromatic amine, mutagenicity, polymerase, SOS functions

Introduction

The carcinogenic and mutagenic effects of the aromatic amines are believed to depend on their covalent modification of DNA, primarily through the formation of adducts at the C8 position of guanine (*1,2*). While adduct formation is likely to be an obligatory event in these processes, the production of adverse biological responses is likely to be influenced by the repair of the lesion (*3*), the need for the cell to utilize the DNA as a template for semiconservative replication (*4,5*), and the bypass of unrepaired lesions by DNA polymerases (*4,5*). Each of these three factors will be affected by the structure of the bound moiety in both qualitative and quantitative ways. The objective of our studies is to attempt to establish the structural characteristics of arylamine adducts that determine their abilities to produce mutagenic effects.

This paper was presented at the Fifth International Conference on Carcinogenic and Mutagenic *N*-Substituted Aryl Compounds held 18–21 October 1992 in Würzburg, Germany.

This study, from the A. Alfred Taubman Facility for Environmental Carcinogenesis, was supported by grant CA45639 from the Department of Health and Human Services, by the National Cancer Institute, and by an institutional grant from the United Way of Detroit.

Address correspondence to C.M. King, Department of Chemical Carcinogenesis, Michigan Cancer Foundation, Detroit, MI 48201. Telephone (313) 833-0715, ext. 383. Fax (313) 833-0504. E-mail charlesk@mcf.roc.wayne.edu

Methodology

Adducts containing 2-aminofluorenyl or 4-aminobiphenyl moieties were synthesized by reaction of guanine-containing oligonucleotides or DNA with *N*-acetoxy-*N*-trifluoroacetyl derivatives—for preparation of the C8-substituted arylamine adducts—or with the *N*-acetoxy-*N*-acetylated aromatic amines to incorporate the arylacetamide group at this position (*3*). Oligonucleotides were reacted with *N*-sulfoxy-*N*-acetyl-4-aminobiphenyl to prepare C8-acetylaminobiphenyl-substituted guanines (*6*). Adduct analyses of DNA (*3*) and oligonucleotides (*4*) were carried out as described. Modified DNA was used to transform bacterial cells by use of a calcium chloride technique (*3,4*). Mutations produced by randomly introduced adducts were detected by a phenotypic selection technique (*7*); mutations induced by single, site-specific adducts were identified by hybridization (*4*). Use of adduct-containing oligonucleotides as templates for DNA polymerases was accomplished by introducing a nonphosphorylated adducted 7-mer into a gapped heteroduplex M13 DNA (*8*). The nicked DNA was linearized with Ava II, denatured, and then primed with an oligonucleotide that would yield a full-length 48-mer product or a 41-mer oligonucleotide with those molecules that had failed to ligate a 7-mer into the gapped heteroduplex DNA. Both DNA polymerase I (large fragment) and AMV reverse transcriptase were used for chain extension (*9*).

Survival and Mutagenicity of Randomly Adducted DNA

Previous studies have shown that, while 2-aminofluorene-substituted double-stranded bacteriophage DNA exhibits a decreased transfection in *uvr*C, but not *uvr*A or *uvr*B Uvr endonuclease deficient mutants, analogous *N*-acetylated aminofluorene adducts are essentially equally sensitive to these three mutants (*10*). Analogous studies with plasmid DNA (pBR322) comparing 4-aminobiphenyl and 2-aminofluorene adducts showed that the 2-aminofluorene C8 guanine adduct was the only one of the four to show minimal reduction in transformation efficiency in *uvr*A and *uvr*B cells (*3*). The D_{37} values of the two biphenyl adducts were essentially equivalent to that of the 2-aminofluorene in *uvr*C; the D_{37} of the acetylated aminofluorene adducts approached a value of one in all three mutants, suggesting that a single adduct was almost totally effective in preventing polymerase bypass. These results demonstrated that the Uvr endonuclease could distinguish between adducts that differed only by the single carbon atom that served to hold the fluorene ring in a more planar configuration than that preferred by the biphenyl moiety in which the two aromatic rings are not coplanar. Furthermore, the *N*-acetyl group of the biphenyl adduct was without apparent effect on polymerase bypass.

Introduction of 2-aminofluorene- and 4-aminobiphenyl-substituted single- or

double-stranded bacteriophage DNA (M13mp9) into the Uvr endonuclease-deficient cells provides insight into the extent of bypass without the possibility that differential excision repair contributed to D_{37} values. The data in Table 1 show that, as expected (*11*), transformation efficiency of adducted single-stranded DNA in *uvr* mutants is no lower than that observed in wildtype cells. In contrast to transfections of double-stranded DNA, the transfection efficiency of acetylaminobiphenyl-substituted single-stranded DNA is far lower than the comparable aminobiphenyl-modified DNA and approaches that of acetylaminofluorene-adducted DNA.

The mutagenicities of the adducted aminobiphenyl DNA preparations in wild-type JM103 cells are shown in Table 2. These data, in agreement with earlier studies with the analogous fluorene adducts (*7,12*), clearly demonstrate that both adducts are mutagenic, the mutagenicity is primarily an SOS-dependent phenomenon, and adducts in single-stranded DNA are much more effective in producing mutations. As compared with the earlier study with 2-aminofluorene adducts (*7,12*), the 4-aminobiphenyl adducts were less mutagenic. The sequence changes in the majority of the mutants produced by the aminobiphenyl and acetylaminobiphenyl adducts occur at guanine (i.e., 41 of 54). However, 10 of the 24 mutants in the subset induced by acetylaminobiphenyl residues in single-stranded DNA were located one or more nucleotides away from a guanine, the only base that had incorporated this moiety.

Table 1. Survival of arylamine-substituted M13mp9 DNA.

Adduct	Host strain	Genotype	D_{37}, adducts/DNA molecule: Single-stranded DNA	D_{37}, adducts/DNA molecule: RF DNA
ABP	JM 103	Wild-type	5.5	34
	AB1886	*uvr*A	5.6	8.5
	AB1884	*uvr*C	5.3	7.0
AABP	JM103	Wild-type	2.0	>34
	AB1886	*uvr*A	2.0	7.0
	AB1884	*uvr*C	2.1	6.5
AF	JM 103	Wild-type	5.0	ND
	AB1886	*uvr*A	5.2	ND
AAF	JM103	Wild-type	0.8	ND
	AB1886	*uvr*A	0.8	ND
	AB1884	*uvr*C	1.0	ND

Abbreviations: ABP, 4-aminobiphenyl; AABP, *N*-acetyl-4-aminobiphenyl; AF, 2-aminofluorene; AAF, *N*-acetyl-2-aminofluorene; ND, not determined.

Mutagenicity of Single, Site-specific Adducted DNA

Studies with oligonucleotides and DNA into which a single adduct had been introduced at a specific site provide the opportunity to experimentally determine the behavior of these adducts in biochemical systems *in vitro* as well as their mutagenic effects *in vivo* (*4,5,13*). Use of DNA constructs that carry replication-inhibiting lesions in the strand opposite the adduct serves to maximize the use of the adducted strand as a template *in vivo* (*4,5*). Identification of mutants by virtue of the inability of clones and plaques to hybridize to oligonucleotide probes under stringent conditions serves to eliminate selection bias in these forward mutation systems (*4,8*). Introduction of fluorene–guanine adducts into a plasmid system incorporating these features demonstrated that the aminofluorene adduct exhibited SOS-dependent mutagenicity, yielding primarily frameshift mutations with loss of the adducted guanine (*4*). The analogous acetylaminofluorene adducts produced no mutations; i.e., they were less than 15% as mutagenic as the deacetylated adduct. Introduction of these adducts into a plasmid that contained uracil in the opposite strand increased the mutagenicity of the aminofluorene by approximately 10-fold, to a mutation frequency of 2.9%. Interestingly, the mutations produced by aminofluorene in the uracil-containing DNA were exclusively base substitutions at the adduct site, primarily G:C to T:A transversions. In contrast to the experiments with the nonuracil-containing DNA, the acetylaminofluorene adducts produced a 0.75% mutation frequency, which included both base substitutions and frameshifts, primarily at sites near to but not at the adduct site.

Virtually all of these mutations were observed in host cells that had been exposed to UV irradiation prior to transformation so as to induce SOS functions. Thus, the mutations are believed to be produced by an active process rather than by passive bypass by a DNA polymerase complex or by the introduction of structural artifacts in constructing the DNA vectors. The differential response to the two adducts clearly illustrates that the mutations are structure-specific. Why mutagenic specificity of the aminofluorene adduct changes when presented to the cell in uracil-containing DNA is not known, but it is interesting to note that this adduct produced only base substitutions upon introduction into human cells (*14*). Attempts to further enhance mutagenesis by transforming Uvr endonuclease-deficient *uvr*A cells (AB1886) produced the unexpected result of a decreased mutagenic response equivalent to that observed in wild-type cells (NM522). Whether this decreased response comes from the absence of a component required for the cells' mutagenic response will require further experimentation. This strain of *uvr*A cells, however, had produced mutagenic responses equivalent to wild-type AB1157 cells in previous studies with acetylaminofluorene-adducted plasmids (*15*).

Effects of Adducts on Polymerases

Experiments designed to explore distinctions between adduct structures, which might be evident by virtue of alterations in the abilities of enzymes to use them as substrates, were patterned on previous studies by Strauss (*16*) and Fuchs (*17*). At the oligonucleotide level, both aminofluorene- (*4,8*) and aminobiphenyl-substituted molecules were readily digested by the exonuclease activity of T4 polymerase (*17*); acetylaminofluorene (*4,8*) and acetylaminobiphenyl derivatives were resistant to digestion. Thus, these susceptibilities did not distinguish between the aryl moities, as was evident in the differences in D_{37} *in vivo* as described previously (*3*) and above.

Use of single, site-specific adducts as templates for DNA polymerases (*13,16*) revealed that both types of fluorene and

Table 2. Mutagenicity of 4-aminobiphenyl and *N*-acetyl-4-aminobiphenyl M13mp9 adducts in JM103 cells.

Adduct	Single-stranded M13mD9 DNA: Adducts/DNA	Single-stranded M13mD9 DNA: % Mutation frequency –SOS	Single-stranded M13mD9 DNA: % Mutation frequency +SOS	Double-stranded M13mD9 DNA: Adducts/DNA	Double-stranded M13mD9 DNA: % Mutation frequency –SOS	Double-stranded M13mD9 DNA: % Mutation frequency +SOS
No adduct	–	0.05	0.054	–	0.013	0.019
AABP	4.3	ND	0.32	15	0.014	0.027
	7.2	0.14	0.37	27	ND	0.062
	9.0	0.11	0.47	31	0.025	0.076
ABP	20	0.045	0.56	29	0.053	0.082
				56	0.061	0.19

Abbreviations: ABP, 4-aminobiphenyl; AABP, *N*-acetyl-4-aminobiphenyl; ND, not determined.

biphenyl adducts could result in hesitation points, as detected on autoradiograms, as well as full-length extension products (Figure 1). The data obtained with AMV reverse transcriptase are shown in Figure 1, but similar data also were obtained with DNA polymerase I (large fragment). The hesitation points corresponded to the base prior to the adduct, the adduct site, and the site 5′ to the adduct. The hesitation points prior to the adduct site were most prominent with the acetylaminofluorene adduct. The aminobiphenyl adduct produced less hesitation than the other three adducts. The acetylaminobiphenyl adduct produced the most prominent hesitation points 5′ to the adduct. These data demonstrate that bypass of each of these adducts can occur and that the presence of the adduct on the template can affect polymerization at positions 5′ to the adduct. Although previous studies with site-specific adducts have demonstrated the polymerase bypass of the aminofluorene adduct, with fidelity (*13*), evidence for the bypass of acetylaminofluorene adducts has been less compelling (*9,18*). Further studies with site-specific modified templates will permit a better understanding of the potential for bypass that appears required for expression of the mutagenicity of these compounds.

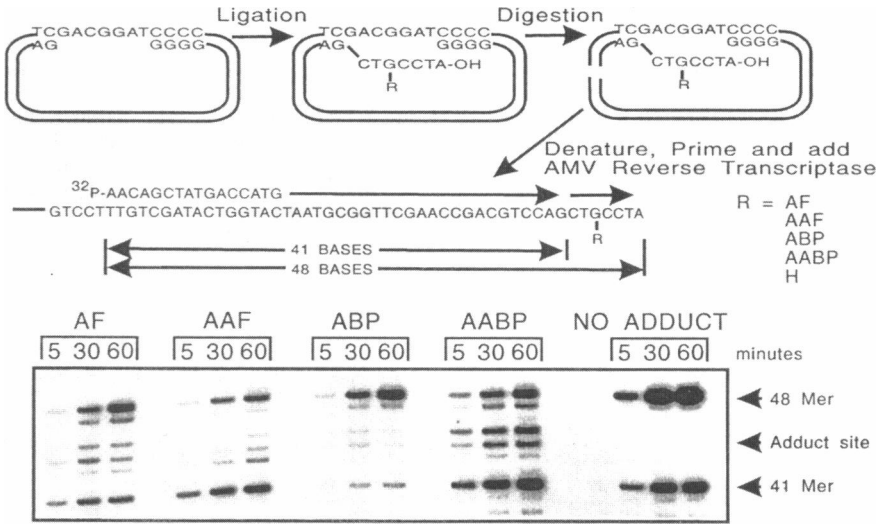

Figure 1. DNA synthesis using site-specific C8 guanine-arylamine adducts as templates.

The *in vitro* nature of the polymerase systems described here are not comparable to those responsible for the production of mutations in the cell. However, they do demonstrate that these macromolecules can, under certain circumstances, distinguish between adducts. It is possible that similar approaches might provide tools to evaluate and identify those structures responsible for the induction of specific mutations in cellular systems.

Conclusions

The studies described here support the idea that aromatic amine adducts at the C8 position of guanine in DNA exert their mutagenic effects because of the specific structures of the adduct, not as a result of faulty repair of the lesion through a general surveillance system. The mutations are produced in bacteria by an active process involving elements of the SOS system. Whether similar systems exist in mammalian cells has not yet been adequately explored. In addition to the specificities of the adduct structures, the mutagenicities of these adducts also are dependent on the sequences into which the adducts are placed and the poorly defined characteristics of these cellular systems involved in the processing of the damaged DNA. Comprehension of these potentials will only come as these systems are identified and characterized.

REFERENCES

1. Beland FA, Beranek DT, Dooley KL, Heflich RH, Kadlubar FF. Arylamine-DNA adducts *in vitro* and *in vivo*: their role in bacterial mutagenesis and urinary bladder carcinogenesis. Environ Health Perspect 49:125–134 (1983).
2. King CM. Metabolism and the "initiation" of tumors by chemicals. In: Arachidonic Acid Metabolism and Tumor Initiation (Marnett LJ, ed). Boston:Martinus Nijhoff Publishing, 1985;1–37.
3. Tamura N, King CM. Comparative survival of aminobiphenyl- and aminofluorene-substituted plasmid DNA in *Escherichia coli* Uvr endonuclease-deficient strains. Carcinogenesis 11:535–540 (1990).
4. Reid TM, Lee M-S, King CM. Mutagenesis by site-specific arylamine adducts in plasmid DNA: enhancing replication of the adducted strand alters mutation frequency. Biochemistry 29:6153–6161 (1990).
5. Koffel-Schwartz N, Maenhaut-Michel G, Fuchs RPP. Specific strand loss in *N*-2-acetylaminofluorene-modified DNA. J Mol Biol 193:651–659 (1987).
6. King CM, Phillips B. *N*-Hydroxy-2-fluorenylacetamide. Reaction of carcinogen with guanosine, ribonucleic acid, deoxyribonucleic acid, and protein following enzymatic deacetylation or esterification. J Biol Chem 244:6209–6216 (1969).
7. Gupta PK, Lee M-S, King CM. Comparison of mutagenesis induced in single- and double-stranded M13 viral DNA by treatment with *N*-hydroxy-2-aminofluorene. Carcinogenesis 9:1337–1345 (1988).
8. Gupta PK, Johnson DL, Reid TM, Lee M-S, Romano LJ, King CM. Mutagenesis by single site-specific arylamine-DNA adducts. J Biol Chem 264:20120–20130 (1989).
9. Moore PD, Rabkin SD, Osborn AL, King CM, Strauss BS. Effect of acetylated and deacetylated 2-aminofluorene adducts on *in vitro* DNA synthesis. Proc Natl Acad Sci USA 79:7166–7170 (1982).
10. Tang M, Lieberman MW, King CM. *uvr* Genes function differently in repair of acetylaminofluorene and aminofluorene DNA adducts. Nature 299:646–650 (1982).
11. Lutgerink JT, Retel J, Westra JG, Welling MC, Loman H, Kriek E. Bypass of the major aminofluorene-DNA adduct during *in vivo* replication of single- and double-stranded ϕX1 74DNA treated with *N*-hydroxy-2-aminofluorene. Carcinogenesis 6:1501–1506 (1985).
12. Gupta PK, Pandrangi RG, Lee M-S, King CM. Induction of mutations by *N*-acetoxy-*N*-acetyl-2-aminofluorene modified M13 viral DNA. Carcinogenesis 12:819–824 (1991).
13. Michaels ML, Reid TM, King CM, Romano L. Accurate *in vitro* translesion synthesis by *Escherichia coli* DNA polymerase I (large fragment) on a site-specific, aminofluorene-modified

oligonucleotide. Carcinogenesis 12:1641–1646 (1991).
14. Mah MC-M, Maher VM, Thomas H, Reid TM, King CM, McCormick JJ. Mutations induced by aminofluorene-DNA adducts during replication in human cells. Carcinogenesis 10:2321–2328 (1989).
15. Koffel-Schwartz N, Verdier J, Bichara M, Freund AM, Daune MP, Fuchs RPP. Carcinogen-induced mutation spectrum in wild-type, *uvr*A and *umu*C strains of *Escherichia coli.* J Mol Biol 177:33–51 (1984).
16. Moore P, Strauss BS. Sites of inhibition of *in vitro* DNA synthesis in carcinogen and UV-treated ϕX174 DNA. Nature 278:664–666 (1979).
17. Fuchs RPP. DNA binding spectrum of the carcinogen *N*-acetoxy-*N*-2-acetylaminofluorene significantly differs from the mutation spectrum. J Mol Biol 177:173–180 (1983).
18. Sahm J, Turkington E, LaPointe D, Strauss B. Mutation induced *in vitro* on a C-8 guanine aminofluorene-containing template by a modified T7 DNA polymerase. Biochemistry 28:2836–2843 (1989).